A comparison of extractives from the bark of *Ekebergia capensis* and *Ekebergia senegalensis*

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Ekebergia capensis and *Ekebergia senegalensis* are considered conspecific. A chemical investigation of the bark and wood of two South African specimens of *E.capensis* has not produced compounds similar to the coumarin, ekersenin, found previously in the timber of a specimen of *E.senegalensis* from Nigeria.

Keywords: *Ekebergia capensis*, *Ekebergia senegalensis*, Meliaceae, medicinal plant, triterpenoids, coumarins.

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Introduction

Ekebergia capensis Sparrm. (Meliaceae) is widely used in traditional Zulu medicine. A decoction of the bark is taken as an emetic for heartburn and for respiratory complaints (Bryant 1966). Ground bark is used in flour and water poultices for abscesses and boils and in hot water infusions for pimples, administered both as a blood purifying emetic and as a wash. Bark is traditionally used to protect chiefs against witchcraft and is also taken in love charm emetics (Gerstner 1941). The Xhosa people use the bark as a disinfectant and to treat heart ailments and infertility (Hutchings *et al.* 1996).

Ekebergia capensis is considered conspecific with the West African *E. senegalensis* (Pennington & Styles 1975). *E. capensis* is one of the most widely used plants in traditional medicine in the Durban area and this work was undertaken in order to determine the chemical composition of the bark and wood of this species.

The hexane extract of the timber of *E. senegalensis* yielded the crystalline compound ekersenin. This compound was originally identified as 4-methoxy-8-methylcoumarin but this structure was shown, on synthesis, to be incorrect and was later revised to 4-methoxy-5-methylcoumarin, **1**, (Bevan & Ekong 1965; Okogun *et al.* 1976). Chemical structures of highlighted compounds are presented in Figure 1. This was the first report of a coumarin from the Meliaceae, but subsequently they have been isolated from *Ekebergia pterophylla* (Mulholland & Iourine, unpublished work) and *Quivisianthe papinae* (Mulholland & Taylor 1988). No triterpenoids were reported by the authors. The hexane and chloroform extracts of bark and wood of two specimens of *E. capensis* were examined in the present investigation in an attempt to isolate ekersenin or related compounds.

Materials and Methods

Two samples of the bark and wood of *E. capensis* were collected. The first came from the Nelspruit Lowveld Botanical Gardens (NG) in the Mpumalanga lowveld and the second from the Silverglen Nature Reserve (SNR) in the Durban area. Samples were authenticated at the Natal Herbarium and voucher specimens retained. Wood and bark from each specimen were dried, milled separately, extracted using a soxhlet apparatus for 24 hours each with refluxing hexane, chloroform then methanol [NG wood (710g) NG bark (225g) SNR wood (147 g) SNR bark (50 g)]. Thin layer chromatography indicated extracts from both sources were the same, except for the presence of one spot, later identified as atraric acid in the NG extract. The NG extracts were separated using gravity column chromatography over silica gel (Merck 9385) using varying proportions of hexane : CH_2Cl_2: EtOAc. Structures were determined by NMR spectroscopy using a Gemini 300 NMR spectrometer and by comparison of data with literature values.

The hexane extract of the bark (NG) yielded atraric acid, **2**, β-sitosterol, **3**, palmitate and oleate esters of β-sitosterol, lupeol, **4**, and oleanonic acid, **5**, The chloroform extract yielded lupeol and the methanol extract yielded 3- *epi*oleanolic acid, **6**. Only lupeol was isolated from the wood. No limonoids nor coumarins were present.

Atraric acid, methyl 2,4 - dihydroxy - 3,6 - dimethylbenzoate **2** (32 mg), m.p. 139–142°C, (lit 140–142°, Dict.Nat.Prods.) ^{1}H NMR ($CDCl_3$, 300 MHz): δ2.08 (3H, *s*, C^3 - CH_3, 2.44 (3H,s, C^6 - CH_3), 3.96 (3H, s, $COOCH_3$), 5.16 (1H, *s*, C^4 - OH), 6.19 (1H, *s*, H-5), 12.00 (lH, *s*, C^2-OH) EIMS: M^+ 196.0745 g. mol^{-1} ($C_{10}H_{12}O_4$ requires 196.0735)

β-*Sitosterol*, 24 - ethylcholest - 5 - en - 3β-ol, **3** (74 mg), m.p. 133–135°C, (lit 135–137°, Dict.Nat.Prods.) ^{1}H NMR δ0.66 (3H, *s*, H-18), 0.78 (3H, *d*, *J* = 7.1Hz, H-27), 0.80 (3H, *d*, *J* = 7.9Hz, H-26) 0.82(3H, *t*, *J* = 7.2Hz, H-29), 0.91 (3H, *d*, *J* = 6.4Hz, H-21), 0.99 (3H, *s*, H19), 3.50 (1H, *m*, H-3), 5.32 (1H, *m*, H-6)
EIMS: M^+ 430 g. mol^{-1}.

β-*Sitosterol oleate and palmitate*, 24 - Ethylcholest - 5 - en - 3 β palmitate / oleate - obtained as a mixture of esters.

Hydrolysis: The mixture (40 mg) was hydrolysed in MeOH saturated with HCl for 1 hour at room temperature. After column chromatography, β-sitosterol and a fatty acid fraction were obtained. GC/MS of the methylated fatty acids gave peaks at m/z 296 (methyl oleate) and 270 (methyl palmitate).

Lupeol, 20- (29) - lupen - 3β - ol, **4** (54.2 mg), m.p. 214–216° (lit 215–216°, Dict.Nat.Prods.), ^{1}H NMR: δ0.76 (3H, *s*, H-23), 0.79 (3H, *s*, H-28), 0.83 (3H, *s*, H-24), 0.94 (3H, *s*, H-27), 0.97 (3H, *s*, H-25), 1.03 (3H, *s*, H-26), 1.68 (3H, *s*, H-29), 3.25 (1H, *m*, H-3), 3.62 (OH), 4.57 (1H, *d*, *J* = 2.4 Hz, H-30a), 4.68 (1H, *d*, *J* = 2.4 Hz, H-30b)
EIMS: M^+ 426 g.mol^{-1}
Oxidation of lupeol with CrO_3/pyridine yielded 20 -(29) - lupen - 3 - one, m.p. 169–170° (lit 168–170°, Dict.Nat.Prods.)

Oleanonic acid, 3 - oxo - 12 - oleanen - 28 - oic acid, **5** (38 mg), m.p. 226–228° (lit 226–229°, Dict.Nat.Prods.) ^{1}H NMR :δ0.75 (3H, *s*, H-26), 0.82 (3H, *s*, H-30) 0.88 (3H, *s*, H-29) 0.98 (3H, *s*, H-24), 0.99 (3H, *s*, H-25), 1.03 (3H, *s*, H-23), 1.09 (3H, *s*, H-27), 2.30 (1H, *m*, H-2 axial), 2.50 (1H, *m*, H-20), 2.78 (1H, *dd*, *J*, = 3.5 Hz, 12.1 Hz, H-18), 5.24 (1H, *br s*, H-12).
EIMS; M^+ 454.3471 ($C_{30}H_{46}O_3$ requires 454.3447)

3-epioleanolic acid, 3α - hydroxy - 12 - oleanen - 28-oic acid, **6** (18.2 mg), m.p. 294–296° (lit 297–299°, Dict.Nat.Prods.) ^{1}H NMR: δ0.73 (3H, *s*, H-26), 0.81 (3H, *s*, H-30), 0.88 (3H, *s*, H-29), 0.90 (6H, *s*, H-24,25), 0.93 (3H, *s*, H-23), 2.79 (1H, *dd*, *J* = 3.9, 13.3Hz, H-18) 3.39 (1H, *s*, H-3β), 5.23 (1H, *m*, H-12).

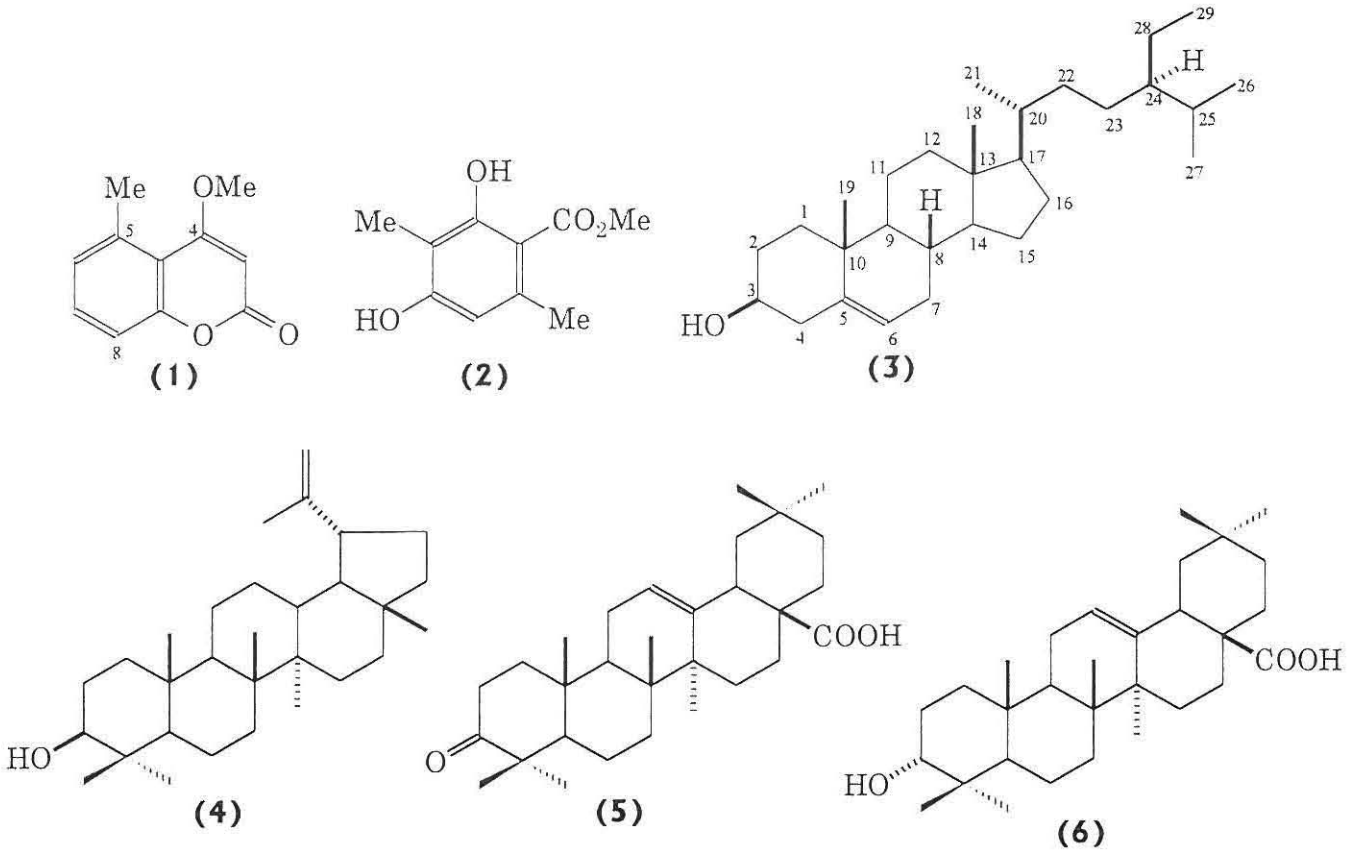

Figure 1 Chemical structures of compounds highlighted in the text.

Results and Discussion

Compounds **3–6** are common triterpenoids found in plants and were identified by comparison of their NMR and other physical data with literature values. The isolation of atraric acid was first thought to be an artefact as it was not detected in the specimen from Silverglen Nature Reserve. This compound has been found to be a major component of the oakmoss, *Evernia prunastri*, although reports have been made of its occurrence in higher plants, for example, the Australian species, *Dianella revoluta*, (Cooke & Down 1971). It is, however, interesting that we have also isolated this compound from the bark extract of *Ekebergia pterophylla* (Mulholland & Iourine, unpublished work). In both cases there was no visible evidence of contamination of the specimen. No coumarins were found in the two specimens investigated.

Pennington and Styles (1975) grouped *Ekebergia senegalensis* and *Ekebergia capensis* together due to their similar morphology. The lack of coumarins in the bark of *E. capensis* could be due to seasonal or regional variation and is not conclusive evidence for disputing the classification of Pennington and Styles (1975).

Oleanolic acid has been proposed for development as an anti-arthritic and anti-inflammatory agent and lupeol has been found to be an anti-arthritic agent (Pettit 1996; Kweifio-Okai *et al*, 1995).

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References

BEVAN, C.W.L. & EKONG, D.E.U. 1965. Occurrence of 8-methoxy -4-methylcoumarin in *Ekebergia senegalensis*. A. Juss. *Chem and Ind.* 383–384.

BRYANT, A.T. 1966. Zulu medicine and medicine men. C. Struik, Cape Town (originally published in 1909 in The Annals of the Natal Museum).

COOKE, R.G. & DOWN J.G. 1971. Colouring matters of Australian plants. *Aus. J. Chem.* 24: 1257.

GERSTNER, J. 1941. A preliminary checklist of Zulu names of plants with short notes. *Bantu Stud.*, 15 (3): 277–301.

HUTCHINGS, A., SCOTT, A.H., LEWIS, G. & CUNNINGHAM, A. 1996. Zulu Medicine Plants, University of Natal Press, South Africa, 157.

KWEIFIO-OKAI, G., FIELD, B. & RUMBLE, B.A. 1995. Esterification improves anti-arthritic effectiveness of lupeol. *Drug Dev. Res.* 35: 137–41.

MULHOLLAND, D.A. & TAYLOR, D.A.H. 1988. Limonoid extractives from the genera *Capurionianthus, Neobeguea* and *Quivisianthe*. *Phytochemistry*, 27: 1741–1743.

OKOGUN, J.I., ENYENIHI, V.U. & EKONG, D.E.U. 1978. Spectral studies on coumarins and the determination of the constitution of ekersenin by total synthesis. *Tetrahedron* 34: 1221–1224.

PENNINGTON, T.D. & STYLES, B.T. 1975. A taxonomic survey of the Meliaceae. *Blumea* 22: 460.

PETTIT, G.R. 1996. Progress in the discovery of biosynthetic anticancer drugs. *J. Nat. Prod.* 59: 812–821.